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# The genus *Buccinanops*: A model for eye loss in caenogastropods*

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**Abstract:** The genus *Buccinanops* (d'Orbigny, 1841) (Caenogastropoda, Nassarriidae) is endemic to the SW Atlantic Ocean, and the name implies *no eyes*, due to the lack of visible eyes in adults. We recognize for the first time the occurrence of eyes during several developmental stages within *Buccinanops*. Eye spots in *Buccinanops cochlidium* (Dillwyn, 1817) were observed during intracapsular development and in hatchlings and juveniles. Eyes were histologically confirmed in embryonic cephalic tentacles; they were comprised of sensory cells, supportive cells, a lens, and an optic nerve cord. The ontogenetic history of the eyes of *B. cochlidium* is discussed.

**Key words:** Nassariidae, eye development, embryology, gastropod blindness, ultrastructure

Molluscs represent a group with a diversity of eye types and range of complexity of eye structure and are becoming of increasing interest when modeling evolution of eye development (Tomarev *et al.* 1997, Arendt 2003, Platcheski *et al.* 2005). In the caenogastropods, a pair of eyes is usually located on the outer side of the cephalic tentacles, embedded in the tentacle or on a small bulge at its base. Originally, a separate stalk contains the eye in the side of the head immediately posterior to the cephalic tentacle (*e.g.*, *Haliotis* Linnaeus, 1758) or partially separated as in the Trochacea (Hyman 1967, Fretter and Graham 1994).

The interest in eye development in gastropods includes topics such as the independent development of the eye in comparison with other classes of molluscs and eye regeneration (Gibson 1984, Bever and Borgens 2005). Even more interesting is the loss of eyes in eyed lineages. The loss of eyes has occurred several times in the gastropods. Within them, some non-related families are cited to have blind representatives such as the archaeogastropod *Pisulina* sp. Neville, 1869 (Neritiliidae) (Kano and Kase 2002), the neogastropods *Buccinanops* sp. (d'Orbigny, 1841) and *Bullia* sp. (Gray in Griffith and Pidgeon, 1834, da Silva and Brown 1985) (both Nassariidae), the opisthobranchs *Retusa* sp. Brown, 1827 (Retusidae) and *Cylichna* sp. Lovén, 1846 (Scaphandridae) (Mikkelsen 2002), and the pulmonates *Cecilioides* sp. de Férussac, 1814 (Ferussaciidae) (Heller *et al.* 1991). A unique and consistent explanation for eye reduction or loss is not agreed upon although it is generally associated with habitual burrowers or living in habitats where light does not reach such as caves or ocean abysses (Hyman 1967, Fretter and Graham 1994, Strickler *et al.* 2001, Kano and Kase 2002). Comparative studies among eyeless species or lineages could help our understanding of why eye reduction and loss occur in nature and complement the modeling of eye development.

The genus *Buccinanops* (Caenogastropoda, Nassarriidae) represents a group of seven species, all endemic to the SW Atlantic Ocean (Pastorino 1993, Rios 1994). The genus name means "*Buccinum without eyes*" due to the lack of visible eyes in the adults (d'Orbigny 1841). Within the genus, *Buccinanops cochlidium* (Dillwyn, 1817) is the largest species (Fig. 1A) and ranges from Rio de Janeiro, Brazil (23°S) to Patagonia, Argentina (42°S). Animals reach up to 110 mm in length and are gonochoristic.

A study of the intracapsular embryological development of *Buccinanops cochlidium* was recently conducted in the field and conditioned aquaria (Averbuj and Penchaszadeh, unpubl. ms). Females of the species attach the egg capsules to the callous region of their own shell. Between 1 and 20 embryos completed their development within the egg capsule after a period of 4 months and the ingestion of thousands of entire nurse eggs. The embryos hatch as 4 mm shelled, crawling juveniles.

Observations made during this study identified small dark spots at the base of the cephalic tentacles of the embryos of *Buccinanops cochlidium* while developing inside the egg capsules. These spots coincided with the description of eye location in the tentacle and aspect of pigmentation (Fretter and Graham 1994). The structure and location of those eyes is studied here. Although we tentatively identified these structures as eyes in the encapsulated embryos, the presence of these structures in juveniles is not confirmed, and they are lacking in the adults (Fig. 1A).

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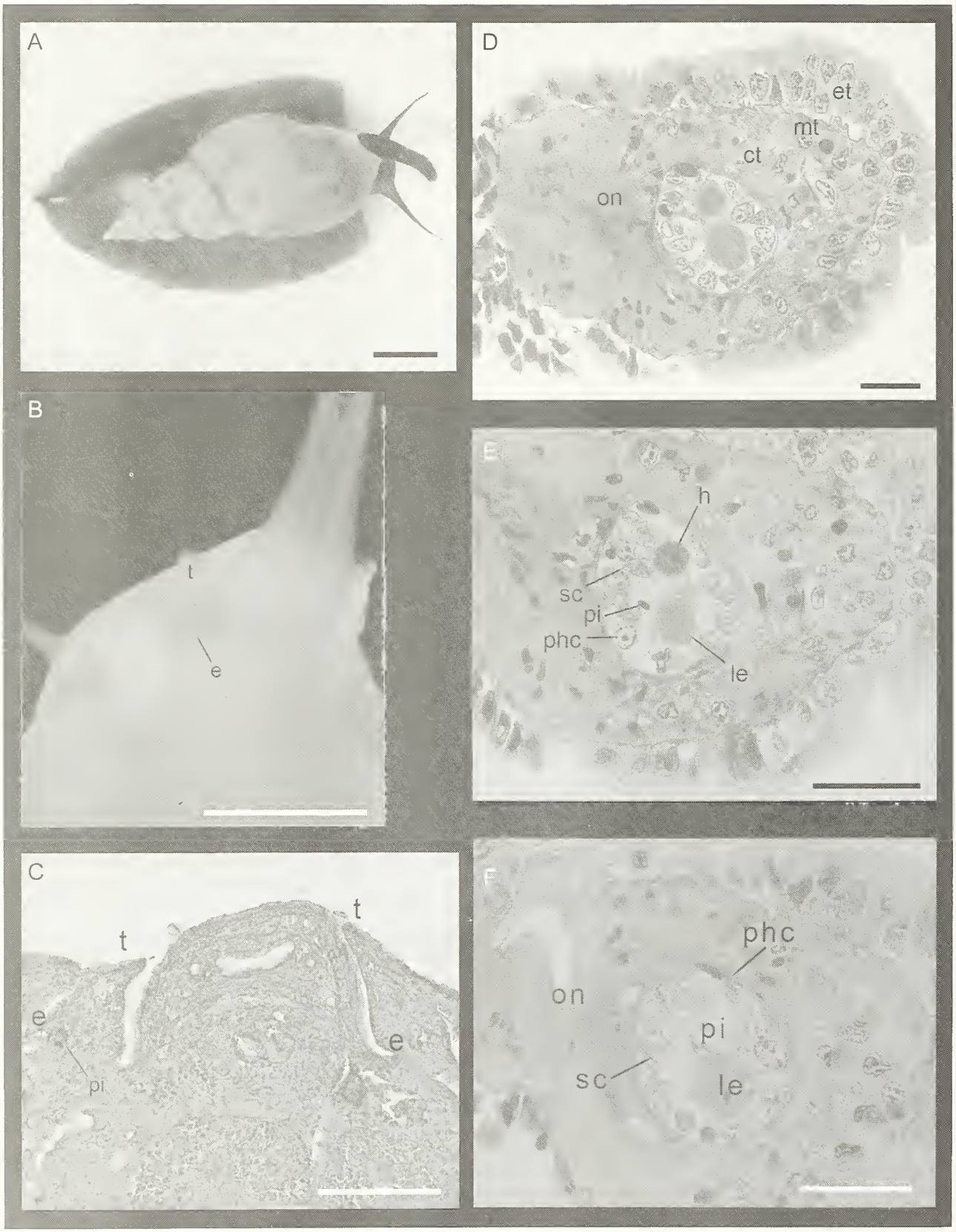
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## MATERIALS AND METHODS

Samples were obtained from Villarino Beach, in San José Gulf, Argentina (42°25′S, 64°31′W). Collection was performed by scuba diving over muddy bottoms at depths varying between 5 and 15 meters. Females with attached egg capsules in different stages of development, juveniles, and adults were collected and taken to laboratory. The animals were maintained in seawater conditioned aquaria until processed. Salinity was fixed at 35 PSU and 12 °C, on a 12-12 h light: dark photoperiod. Individuals' shell length was measured with 0.1 mm precision vernier calipers.

Egg capsules representing all stages of embryonic development were detached from the shell of gravid females and dissected. Total shell length (TSL) of the embryos and free living individuals were measured at different stages of development (modified from Bigatti 2005) defined as: cell division, morulae, "veliger", late "veliger", coiling, pre-hatching, hatchling (all inside the capsules), juveniles, and adults. All measurements were made under a Zeiss stereoscopic microscope with a 0.1 mm precision ocular micrometer. Using a microscope, presence or absence of eyes was recorded at each of the different developmental stages as well as in hatchlings, juveniles, and adults. Whenever eyes were found, the cephalic tentacles containing the eye were dissected for histology studies. When the eyes were not visible, the whole tentacle was fixed and preserved for continuous sectioning.

Material was pre-fixed in 2.5% glutaraldehyde for two hours and rinsed in sodium cacodylate buffer, post-fixed with 2% Osmium for 1 hour, and rinsed again in sodium cacodylate buffer. Fixed specimens were serially dehydrated with ethanol in graded steps and embedded in Spur's epoxy resin. Sectioning of embedded specimens was done in 1 μm sections from the base of the tentacle to the apex. Slides were stained with Methylene Blue for optical microscopy.

When possible, sections of the eye were mounted on TEM copper grids and stained with 2% uranyl acetate (Reynolds 1963). This technique enabled the visualization of microvilli and/or cilia used to define cell types.

## RESULTS

Eye spots were first recognized in all intracapsular embryos at a late "veliger" stage (2.9 ± 0.5 mm of total length; $N = 39$) when the cephalic tentacle is developed conspicuously and the foot and shell have already started to develop. Eyes were also observed at the pre-hatching stage (3.5 ± 0.44 mm; $N = 38$) and in hatchlings (4.0 ± 0.6 mm of total shell length; $N = 626$; Fig. 1B).

The eye is located at the basal region of the cephalic tentacles (Fig. 1B-C). In transverse section of the tentacle, the structure of the eye shows a basal membrane, a retina, and a lens (Fig. 1D-E). Two cellular types (probably photoreceptor and supportive cells) appear to be present and forming in the retina. One cell type shows condensed chromatin (euchromatin) which is observed as dark nuclei and corresponds to the photoreceptor cells. The supportive cells, which occur in a higher frequency than the photoreceptor cells, have less condensed chromatin (heterochromatin; Fig. 1F).

Pigmentation is present in both cell types in different degrees of density. The eye's maximum width in the embryos ranged between 35 and 40 μm ($N = 8$), in embryos measuring from 2 to 5 mm of total length. We could not identify an area for entrance of light to the eye in any section of the tentacles, as each eye was consistently surrounded by tentacle tissues (epidermal, muscular, and connective tissues; Fig. 1D).

The black spots were also recognized macroscopically in a single 15 mm crawling juvenile (total shell length), but we could not find an eye structure microscopically. Tentacles of an adult (60 mm of TSL) were also studied microscopically but no eye was found.

## DISCUSSION

In this work we recognized for the first time the presence of eyes in the encapsulated late embryo and confirmed them histologically in late intracapsular embryo stages. Although there certainly are other species of snails without eyes in the adult individuals (in Argentina the genus *Olivancillaria* d'Orbigny, 1841 (Olividae) and other groups cited above) but where embryos probably have eyes, to our knowledge this is the first study on the embryonic eyes of a blind gastropod species. Loss or reduction of eyes is usually associated with living in poorly illuminated environments

**Figure 1.** *Buccinanops cochlidium*. A, Adult specimen of *B. cochlidium* from Villarino Beach, Patagonia. B, Dark spots at the base of the tentacles of a pre-hatching embryo. C, Cross section of the cephalic region of a late "veliger" embryo. The eye on the left tentacle is pigmented. D, Cross section at the base of the tentacle. E, Detail of the eye with lens and humor and surrounded by tissues (connective, muscular, and epidermal). The optic nerve is shown. F, Detail of the eye at the base of the tentacle of a pre-hatching embryo, with a unique lens and different cell types. Abbreviations: e, eye; t, tentacle; on, optic nerve; le, lens; h, humor; pi, pigmentation; phc, photoreceptor cell; sc, supportive cell; ct, connective tissue; mt, muscular tissue; et, epidermal tissue. Scale bars = 2 cm (A), 1 mm (B), 100 μm (C), and 20 μm (D-F).

(Hyman 1967, Fretter and Graham 1994). In this case, *Buccinanops cochlidium* lives in shallow waters in a well-illuminated environment, yet individuals often are found to be buried a few centimeters in the muddy/sandy bottom. An exception to this observation is when the animals are feeding isolated or in groups on carrion.

Two cellular types appear to be present in the retina of *Buccinanops cochlidium*. Pigmentation is present in both types, but in aggregations of different densities. A lens, a basal membrane, and an optical nerve complete the structure of the photoreceptor organ. The lens sometimes appears as one big roundish structure, while in other cases it is smaller and accompanied by a second similar structure which is colored darker, resembling a humor (Fig. 1E).

Although a pair of dark spots was observed in a 15 mm (post-hatched) individual, it was not possible to confirm histologically the presence of eyes. Studying individuals in this size range would be important to determine whether the eye is conserved intact or modified, deeply embedded in the tentacle tissue, or if it degenerates as the animal ages. In snails measuring more than 20 mm of TSL, eye spots are not visible macroscopically; thus, the hypothesis that eyes degenerate in adults is a strong possibility. How does the degeneration occur? Kano and Kase (2002) discussed possibilities such as reduction in size, loss of retinal pigmentation, or sinking under the skin.

At the moment, TEM techniques are being used to complete ultrastructure information of the embryonic eyes and attempt to confirm the photoreceptor cell type, rhabdomeric or ciliary (Arendt 2003, Plachetzki *et al.* 2005). Tentacles of juvenile and adult individuals of increasing size ranges were preserved for later studies (continuous serial cut). If eyes occur in juveniles or adult snails, comparison of the ultrastructure with that of the embryo will be relevant in order to know whether it remains equal or if it is modified in type and number of retinal cells, as happens in other snails (Blumer 1996, 1998). We found no literature about modification or loss of embryonic eyes in species with no adult eyes. Additional work comparing the structure and function of *Buccinanops* eyes in related groups, such as the buccinid *Buccinum* sp. (Linnaeus, 1758) or the nassariid *Bullia* sp. (Brown 1982, Cernohorsky 1984, Allmon 1990) (with and without eyes, respectively) may also be insightful.

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